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EXAMINER	
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ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/529,654	VEDRINE ET AL.	
	Examiner	Art Unit	
	OLUWATOSIN OGUNBIYI	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 May 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-10,12,13 and 15-22* is/are pending in the application.

4a) Of the above claim(s) *18-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10,12,13,15-17,21 and 22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/16/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/7/2010 has been entered.

2. Claims 1-2, 4, 7, 21 and 22 are amended. Claims 1-10, 12-13 and 15-22 are pending in the application. Claims 1-10, 12-13, 15-17, 21 and 22 are under examination. Claims 18-20 are withdrawn.

Information Disclosure Statement

3. The information disclosure statement filed 6/16/10 has been considered and an initialed copy is enclosed.

Claim Rejections-Withdrawn

4. The rejection of claims 11 and 14 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 (cited in IDS) in view of Pyle et al WO 95/31481 23 November 1995 (cited in IDS) is withdrawn in view of the cancellation of the claims.

5. The rejection of claims 2, 3 and 22 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement (new matter) is withdrawn in view of Applicants explanation that in the international application, the "u" means unit and that IU simply refers to international Unit.

6. The rejection of claims 7, 11-14, 16 and 22 under 35 U.S.C. 112, second paragraph, is withdrawn in view of the amendment to the claims.

Rejections Maintained

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. The rejection of claims 2-3 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, is maintained.

The term *about 5000 IU/L* in claims 2-3 is a relative term which renders the claim indefinite. The term *about* is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention e.g. the upper or lower limits of *about 5000 IU/L.*

Applicants' arguments and the response:

Applicants traverse the rejection and in further response state that Applicants have deleted this term thereby rendering this objection moot. This is not persuasive. The term " *about 5000 IU/L"* in claims 2-3 renders the metes and bounds of the claims indefinite for the reasons set forth in the rejection above. Furthermore, the term is still a limitation in claims 2 and 3 and has not been deleted. Therefore the instant rejection still applies.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. The rejection of claims 1, 4-6, 15 and 17 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 (cited in IDS) in view of Pyle et al WO 95/31481 23 November 1995 (cited in IDS) is maintained.

The claims are drawn to a method for detecting and counting intracellularly labeled microorganisms in a sample comprising the steps of:

a) selectively enriching the microorganism sought in the sample,

b) inducing or activating at least one enzymatic activity of the microorganism,

c) immunomagnetically concentrating the
microorganism,

d) fluorescently labeling the microorganism by adding to the sample containing the microorganism at least one substrate comprising one part specific to the enzymatic activity to be indicated and one fluorogenic label, wherein the transformation of the substrate takes place inside the microorganism and wherein the fluorescent product resulting from the fluorogenic label is retained in the microorganism, and

e) detecting and counting the fluorescene labeled
microorganisms by fluorescence microscopy.

Berg et al teaches a method of assaying a dilute concentration of living pathogenic microorganisms in a sample of product for human consumption, comprising: Step a) contacting a sample comprising microorganisms e.g. bacteria with a nutrient medium capable of supporting the metabolism and reproduction of the microorganisms (selectively enriching the microorganisms sought in the sample), see p. 6 lines 5-8

Step b) inducing the production of an enzyme (e.g. lipase or esterase or B-D-galactosidase see p. 7 lines 13-18, p. 8 lines 1-7) in said microorganism by contacting said microorganism with an agent such as lactose that is capable of inducing the production of an enzyme (specific to the microorganism sought) in said microorganism (inducing or activating at least one enzymatic activity), see p. 6 lines 5-10 step 2

Step d) contacting by adding to the media containing the enzymatically induced microorganism a fluorogenic substrate (e.g. 4-methylumbelliferone-heptanoate or 4-methylumbelliferone-B-D-galactosidase, see p. 7 lines 18-24, p. 8 lines 1-7) which reacts with the enzyme (one part specific to the enzymatic activity) to release the fluorescent portion thereof (fluorogenic label part which comprises 4-methylumbelliferone (see p. 7 lines 13-18), wherein the transformation of the substrate takes place inside the microorganism and the fluorescent product resulting from the fluorogenic label is retained in the microorganism. See p. 6 lines 5-20. Said fluorescent product is retained in the microorganism as Berg et al specifically teaches contacting of the enzyme inside the microorganism with the fluorogenic substrate and release of the fluorescent portion of the fluorogenic substrate *from each single microorganism* and its descendants to form a visible microcolony under fluorescent conditions. See p. 9 lines 1-15

Step e) Berg et al teaches counting of the number of fluorescent microcolonies. See p. 6 - 7, p. 9 lines 14-15 and p. 21-22 claim 1.

Berg et al teaches that the method of detecting and counting intracellularly labeled microorganisms in a sample makes use of the enzyme activity in living microorganisms to detect their presence and to quantify them. See p. 11 lines 1-6.

Berg et al teach that the addition of a permeability agent is optional as Berg et al teach an example using the above steps a, b, d and e to detect and count intracellularly labeled microorganisms in a sample without the permeability agent. See example 1 p. 14-17 and figure 1 open circle (with lactose which induces the enzyme in the microorganism) and figure 1 (triangles with lactose and permeability agent which enhances the fluorescence of the microorganism)

As to claim 5, Berg et al teaches that selectively enriching the microorganism and inducing or activating the enzymatic activity can be carried out simultaneously since Berg et al teaches that the microorganisms are initially contacted with an actuating medium that comprises 1) the nutrient media which selectively enriches the microorganism and 2) the production agent e.g. lactose which is the enzyme inducer. See p. 6 lines 5-15.

Berg et al teaches that the fluorescent labeling of the microorganism is carried out by adding to said actuating media the fluorogenic substrate which comprises a substrate part specific to said enzymatic activity and a fluorescent label part (e.g. fluorogenic substrates: 4-methylumbeliferone-heptanoate p. 7 lines 19-24, or 4-methylumbeliferone-beta-D-galactoside, p. 14 line 20-25, wherein cleavage by induced enzymes reveals fluorescent 4-methylumbeliferone). See p. 6 lines 5-15.

As to claim 15 and 17, Berg et al teaches that the sample can be filtered before commencing on the steps of detecting and counting the microorganism e.g. see p. 12 for 0.2 um to 0.8 μm pore size, p. 14 example 1 lines 11-14 using filter pore size 0. 045 μm, p. 19 lines 8-10.

Berg et al does not teach step c i.e. immunomagnetically concentrating the microorganisms and does not teach immunomagnetic concentration of the enzymatically activated microorganism after the fluorescent labeling of the microorganism, and does not teach immunomagnetic concentration of the microorganisms before inducing enzymatic activity.

Pyle et al teaches a method for detection and enumeration of viable microorganisms in a sample. Pyle et al also relies on a metabolic indicator and uses immunomagnetic separation/concentration using antibodies which specifically binds to a target bacteria to concentrate the bacteria before addition of a metabolic indicator and before detection of fluorescence. See p. 38 lines 1-29. Pyle et al teaches that immunomagnetic separation is a widely used method for facilitation concentration and separation from samples (see p. 38 lines 30 to p. 40) and that immunomagnetic capture not only permits cell concentration but also the selection of a specific antigenic cell type. See p. 41 lines 7-19.

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to have immunomagnetically concentrated the enzymatically induced microorganisms of Berg et al before addition of the fluorogenic substrate in order to concentrate a specific antigenic cell type in the sample prior to the fluorescent labeling and counting as taught by Pyle et al (p. 38 lines 30 to p. 40). The motivation to do so is provided by Pyle et al who teaches that immunomagnetic capture not only permits cell concentration but also the selection of a specific antigenic cell type), thus resulting in the instant invention with a reasonable expectation of success.

As to claim 6, it would have been prima facie obvious at the time the instant invention was made to immunomagnetically concentrate the microorganisms in the sample before the enriching step or enzyme induction (immunomagnetic concentration before induction or activation of enzymatic activity) or immunomagnetically concentrating the microorganisms after fluorescent labeling, so as to concentrate a specific antigenic cell type, thus resulting in the instant invention with a reasonable expectation of success. The motivation to do so is provided by Pyle et al who teaches that immunomagnetic capture/concentration not only permits cell concentration but also the selection of a specific antigenic cell type (see p. 41 lines 7-19) and there would have been a reasonable expectation of detecting and counting microorganisms irrespective of when specific antigenic type of microorganism is concentrated or captured from the sample.

Applicants' arguments:

Applicants argue Berg et al and that Applicants disagree with the interpretation of the claims by the Examiner which is that the claims do not recite "strictly intracellular labeling of the microorganisms", that the claims recite "comprising" so as not to exclude labeling at other sites" and that Berg "gives the option of contacting the enzyme inside or outside of the microorganism with a fluorogenic substrate and Applicants disagree with the Examiner's interpretation of Berg.

Applicants state that is it is clear from part d) of claim 1 that the label occurs and is retained within the cell. Applicants' statement is carefully considered but is not persuasive. It is the Office's position that the part d) of claim 1 does not merely teach that the label occurs and is retained within the cell. Instead part d of claim 1 recites that the at least one substrate comprising one part specific to the enzymatic activity to be indicated and one fluorogenic label is **added** to the sample containing the microorganism. Claim 1 part d further indicates that "the transformation of the substrate takes place inside the microorganism and that the fluorescent product resulting from the fluorogenic label is retained in the microorganism".

Berg et al et al teach that the fluorescent labeling of the microorganism is carried out by adding to said actuating media the fluorogenic substrate which comprises a substrate part specific to said enzymatic activity and a fluorescent label part (e.g. fluorogenic substrates: 4-methylumbeliferone-heptanoate p. 7 lines 19-24, or 4-methylumbeliferone-beta-D-galactoside, p. 14 line 20-25, wherein cleavage by induced enzymes reveals fluorescent 4-methylumbeliferone). See p. 6 lines 5-15.

Berg et al teach that the addition of the permeability agent is optional as Berg et al teach an example using the above steps a, b, d and e to detect and count intracellularly labeled microorganisms in a sample without the permeability agent. See example 1 p. 14-17 and figure 1 open circles (with lactose which induces the enzyme in the microorganism) and figure 1 (triangles with lactose and permeability agent which enhances the fluorescence of the microorganism) and figure 2 (showing cells counted by fluorescence microscopy). Said fluorescent product is retained in the microorganism as Berg et al specifically teaches contacting of the enzyme inside the microorganism with the fluorogenic substrate and release of sufficient of the fluorescent portion of the

fluorogenic substrate *from each single microorganism* and its descendants to form a visible microcolony under fluorescent conditions. See p. 9 lines 1-15

Applicants state that the intracellular nature of the labeling of microorganisms in accordance with the claimed method is relevant and that the intracellular labeling permits the counting of the fluorescently labeled microorganisms by a technique selected from the group consisting of flow cytometry, filtration cytometry, and fluorescent microscopy and that these techniques allow the detection of the fluorescence of each fluorescent cell. Accordingly, the background fluorescence is not registered, thus improving sensitivity and specificity.

Applicants' statement is carefully considered but is not persuasive. The combination of Berg et al and Pyle et al renders the instant claims obvious as set forth in the above rejection. Furthermore, the combination teaches that the method of detecting and counting intracellularly labeled microorganisms in a sample makes use of the enzyme activity in living microorganisms to detect their presence and to quantify them. See Berg et al p. 11 lines 1-6. The combination teaches that said fluorescent product is retained in the microorganism as Berg et al specifically teaches contacting of the enzyme inside the microorganism with the fluorogenic substrate and release of sufficient of the fluorescent portion of the fluorogenic substrate *from each single microorganism* and its descendants to form a visible microcolony under fluorescent conditions. See p. 9 lines 1-15.

Furthermore, the instant claims do not specify that background fluorescence is not registered in order to improve sensitivity and specificity and thus is not commensurate with the scope of the claims. Although, USPTO personnel are to give claims their broadest reasonable interpretation in light of the supporting disclosure (In re Morris, 127 F.3d 1048, 1054-55, 44 USPQ2d 1023, 1027-28 (Fed. Cir. 1997)), limitations appearing in the specification but not recited in the claim should not be read into the claim. E-Pass Techs., Inc. v. 3Com Corp. , 343 F.3d 1364, 1369, 67 USPQ2d 1947, 1950 (Fed. Cir. 2003) (claims must be interpreted "in view of the specification" without importing limitations from the specification into the claims unnecessarily).

Applicants further argue that the Examiner believes that "Berg gives the option of contacting the enzyme inside or outside of the microorganism, with a fluorogenic

substrate but that the claims are directed to strictly intracellular labeling and that Berg does not disclose or suggest strictly intracellular labeling and the association of such labeling with numeration or counting of microorganisms. Applicants' arguments are carefully considered but are not persuasive.

It is the Office's position that the claims are drawn to detecting and counting intracellularly labeled microorganisms and not directed to "strictly intracellular labeling". The combination of Berg et al and Pyle et al teaches step a) to e). Berg et al teaches intracellular labeling of the microorganisms and counting of fluorescene labeled microorganisms which obviously will have the fluorogenic label retained in the microorganism. The combination of Berg et al and Pyle et al detects and counts the fluorescent microorganisms.

The Office has carefully considered but disagrees with Applicants' statements that Berg does not disclose the retention of the label with the cell and that the fact that Berg may suggest that the substrate contacts the enzyme inside or outside the cell microorganism is not the same thing as retaining the label within the cell.

It is the Office's position that Berg et al specifically teaches contacting of the enzyme inside the microorganism with the fluorogenic substrate and release of the fluorescent portion of the fluorogenic substrate *from each single microorganism* and its descendants to form a visible microcolony under fluorescent conditions. See p. 9 lines 1-15.

Berg et al teaches the method of detecting and counting intracellularly labeled microorganisms in a sample and makes use of the enzyme activity in living microorganisms to detect their presence and to quantify them. See p. 11 lines 1-6.

As to the use of a permeability agent, Berg et al teach that the addition of the permeability agent is optional as Berg et al.

As to Applicants arguments that these permeability agents can cause lysis of the cells, this is mere speculation by Applicants and nothing in Berg et al suggests that the permeability agents lysed the cell. In fact, Berg et al teach that the addition of lactose (enzyme inducer) and sodium lauryl sulfate (permeability agent) actually enhanced galactosidase activity within the microorganism. See p. 16 lines 9-22.

Applicants' arguments that these permeability agents can cause lysis of the cells and thus Berg's methods teach away from what Applicants claim i.e. the retention of intracellular label is not persuasive because Berg et al actually counts the fluorescent colonies. See p. 6 -7, p. 9 lines 14-15 and p. 21-22 claim 1, figure 1 and figure 2.

Furthermore, it is obvious that sodium lauryl sulfate did not lyse the microorganisms because Berg et al teach that sodium lauryl sulfate is a common selective ingredient for coliform bacteria and further enhanced the activity of B-D-galactosidase enzyme within the coliform bacteria (see p. 16 lines 1-7) and teaches that galactosidase activity was attributable to the live coliform group (see p. 16 lines 8-22).

Moreover, Berg et al teach an example without the permeability agent. See example 1 p. 14-17 and figure 1 open circle (with lactose which induces the enzyme in the microorganism) and figure 1 (triangles with lactose and permeability agent which enhances the fluorescence of the microorganism).

Applicants' arguments that the method disclosed in Berg necessitates a series of measurements and comparison with a standard curve to determine the concentration of microorganisms in the sample is not persuasive. The combination of Berg et al and Pyle et al teach a method of detecting and counting intracellularly labeled microorganisms in a sample comprising step a)-step e) as set forth in the instant claims and the instant method does not exclude other method steps.

Applicants' arguments that the released fluorescence in the medium creates a background fluorescence which does not permit a specific numeration or counting of the microorganism is carefully considered but is not supported by any evidence. Furthermore, even if there is background fluorescence the intracellularly labeled microorganisms are still detected and counted and the combination of Berg et al and Pyle et al teaches detecting and counting the number of fluorescent microorganisms

Applicants' argument that the method of claim 1 relates to counting each microorganism whereas Berg's method relates to quantification of a fluorescent signal is not persuasive. Berg et al teaches that one simply counts the number of fluorescent microcolonies (see p. 9 lines 14-15) thus the combination of Berg et al and Pyle et al teaches detecting and counting the number of fluorescent microorganisms.

Applicants argue that Pyle does not disclose or suggest a step of selectively enriching the microorganism sought in the sample, nor does it disclose or suggest the use of a substrate comprising one part specific to the enzymatic activity, nor does it disclose or suggest the induction/activation of at least one enzymatic activity of the microorganism and nowhere does Pyle disclose or suggest the strictly intracellular labeling that is required for the numeration and counting microorganisms, according to the claimed invention. Applicants also argue that likewise, the other cited art, either alone or in combination with Berg and Pyle et al, fail to teach or suggest these claimed features of the invention.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The combination of Berg et al and Pyle et al teaches a method for detecting and counting the number of intracellularly labeled microorganisms as set forth in the rejection above.

In view of the above rejections and the response to Applicants arguments above, the rejection is maintained.

9. The rejection of claim 16 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 and Pyle et al WO 95/31481 23 November 1995 as applied to claims 1, 4-6, 15 and 17 above, further in view of Sigma catalog 1996 p. 2179-2181 is maintained.

The combination of Berg and Pyle is set forth supra. Said combination does not teach a filtration step before a), b) c), d) and e) using a filter whose porosity size is 20-100 microns.

Sigma catalog teaches filters with various porosity size (retention size) including between 20-25 and 30 microns. See p. 2181.

Since the combination of Berg and Pyle et al teach detection of microorganisms in water comprising sewage effluent or raw domestic sewage (see Berg et al, example 1, p. 1), it would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to filter the water sample containing sewage using a filter with large pore size e.g. 20-25 or 30 microns or larger (see Sigma catalog p. 2181) to remove any particulate matter prior to enriching microorganisms in the sample, and the motivation would have been to aid in the detecting and counting process and thus resulting in the instant invention with a reasonable expectation of success.

Applicants' arguments:

Applicants traverse this rejection for the same reasons set forth above in connection with the rejection over Berg and Pyle as combined.

Response:

Applicants' arguments are carefully considered but are not persuasive. The rejection of Berg in view of Pyle et al is set forth above. Applicants arguments with respect to the combination of Berg et al and Pyle et al have been carefully considered, are not persuasive and have been addressed above.

10. The rejection of claims 8-10 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 and Pyle et al WO 95/31481 23 November 1995 as applied to claims 1, 4, 5, 6, 15 and 17 above, further in view of Olsen et al. Plant and Soil 186:75-79, 1996 is maintained.

The combination of Berg and Pyle is set forth supra. Said combination does not teach (claim 8) that the immunomagnetic concentration step comprises a) placing the microorganism sought, present in the sample, in contact with an antibody directed against an antigen specific to the microorganism, the antibody being conjugated with a magnetic bead, b) separating the bead-antibody-microorganism complexes from the sample, and c) separating the microorganism from the rest of the complex; does not teach (claim 9) a method according to claim 8, wherein the antibody conjugated with a magnetic bead is directed against an antibody that is itself directed against an

antigen specific to the microorganism sought; does not teach (claim 10) a method according to claim 8 or 9, wherein the magnetic beads have a diameter that is between 1 and 20 um, or between 2 and 8 um.

Olsen et al teaches a method of detecting and counting the number of bacteria in sample using immunomagnetic concentration before detecting and counting of the concentrated cells by fluorescence microscopy. See p. 77 column 1 and column 2 figure 2. The immunomagnetic procedure uses magnetic beads that are 2.8 um in diameter, p. 76 column 2 under immunomagnetic beads, and comprises contacting the bacteria in the sample with an antibody directed against the bacteria, separating the bead antibody bacteria complexes from the medium and separating the microorganism from the rest of the complex by vortexing to detach the beads and separation of the beads via magnetization (see p. 77 column 1 under analytical procedure). Said magnetic bead also comprises a secondary antibody that is directed to the antibody that is directed to the bacteria, which aids in detection of the bacteria.

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to carry out the immunomagnetic concentration step of Berg et al and Pyle et al as combined using the known immunomagnetic concentration guideline set forth in Olsen et al which involves contacting the bacteria in the sample with an antibody directed against the bacteria being sought, separating the bead antibody bacteria complexes from the medium and separating the microorganism from the rest of the complex by vortexing to detach the beads and separation of the beads via magnetization procedure and using magnetic bead comprising a secondary antibody that is directed to the antibody that is directed to the bacteria, which aids in detection of the bacteria, (see p. 77 column 1 under analytical), thus arriving at the instant invention with a reasonable success. The combination of Berg et al and Pyle et al teach a method of detecting and counting bacteria in a sample using the combination of immunomagnetic separation and fluorescence microscopy and Olsen et al also teaches a method of detecting and counting the number of bacteria in sample using immunomagnetic concentration band detecting and counting of the concentrated cells by fluorescence microscopy, it would have been prima facie obvious to adapt the general

immunomagnetic concentration protocol set forth in Olsen et al and tailor said protocol for the immunomagnetic concentration step in Berg and Pyle et al as combined as both immunomagnetic concentration techniques are essentially accomplishing the same thing i.e. concentration of a specific antigenic type of bacteria from a sample.

Applicants' arguments:

Applicants traverse this rejection for the same reasons set forth above in connection with the rejection over Berg and Pyle as combined.

Response:

Applicants' arguments are carefully considered but are not persuasive. The rejection of Berg in view of Pyle et al is set forth above. Applicants arguments with respect to the combination of Berg et al and Pyle et al have been carefully considered, are not persuasive and have been addressed above.

11. The rejections of claims 12 and 13 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 and Pyle et al WO 95/31481 23 November 1995 as applied to claims 1, 4,5,6, 15 and 17 above, further in view of Boyd et al US 5,510,243 Apr. 23 1996 is maintained.

The combination of Berg and Pyle is set forth supra. Said combination does not teach the use of a monosaccharide substrate part specific to the enzymatic activity to be revealed and does not teach that the fluorogenic label is a xanthene.

Boyd et al teaches the method of detecting bacteria *E. coli* and discriminating between said *E. coli* and non-target bacteria using fluorogenic substrates such as fluorescein-di-beta-D-galactopyranoside which has a label part that is a xanthene i.e. fluorescein and a substrate part (di-beta-D-galactopyranoside) comprising a monosaccharide which is specific to Beta-galactosidase enzyme and Boyd et al teaches the use of galactosidase inducers e.g. IPTG to include in media comprising the *E. coli* to be detected. See column 3 line 14-16, 50 to 667 to column 4 lines 1-6 and 60- 67.

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to substitute the fluorogenic substrate (which

comprises a substrate part and label part) and the enzymatic inducer of the combination of Berg and Pyle with another fluorogenic substrate and inducer known in the art for detection of bacteria in a sample such as fluorescein-di-beta-D-galactopyranoside which has a label part that is a xanthene i.e. fluorescein and a substrate part (di-beta-D-galactopyranoside) comprising a monosaccharide which is specific to Beta-galactosidase enzyme and the galactosidase inducer e.g. IPTG (see Boyd et al column 3 line 14-16, 50 to 67 to column 4 lines 1-6 and 60- 67), thus resulting in the instant invention with a reasonable expectation of success. It would have prima facie obvious to substitute one known fluorogenic substrate and inducer system for another as they are similarly used for the same thing i.e. detecting bacteria in a sample.

Applicants' arguments:

Applicants traverse this rejection for the same reasons set forth above in connection with the rejection over Berg and Pyle as combined.

Response:

Applicants' arguments are carefully considered but are not persuasive. The rejection of Berg in view of Pyle et al is set forth above. Applicants arguments with respect to the combination of Berg et al and Pyle et al have been carefully considered, are not persuasive and have been addressed above.

12. The rejection of claim 7 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 and Pyle et al WO 95/31481 23 November 1995 as applied to claims 1, 4, 5, 6, 15 and 17 above, further in view of Kaclikova et al. Journal of Microbiological Methods, Vol. 46 Issue 1 July 2001, p. 63-67 is maintained.

Berg el and Pyle et al as combined is set forth supra. The combination does not teach addition of yeast extract to the enrichment medium.

Kaclikova et al teaches a method of detecting *Listeria* in a product for human consumption (cheese) using enrichment media (Fraser broth) and immunomagnetic separation. See p. 63 column 2 lines 1-3 and p. 64. under section 2.4. Half-Fraser broth comprises amongst other things yeast extract 5g/L[1].

Since the combination of Berg and Pyle is drawn to detection and counting of pathogenic microorganisms in a sample of product for human consumption (see abstract of Berg et al), it would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to modify the enrichment media of Berg and Pyle as combined by adding nutrients for enriching and detecting other pathogenic microorganisms in products for human consumption such as *Listeria*. The motivation to do so is because Kaclikova et al teaches that Listeria can also be detected in food samples (see title) and that nutrients for enriching Listeria include those contained in Half-Fraser which comprises yeast extract (see Kaclikova et al p. 63 column 2 lines 1-3 and p. 64. under section 2.4., thus resulting in the instant invention with a reasonable expectation of success.

[1] Technical Bulletin for Fraser Listeria Enrichment Broth Base: for the selective enrichment of Listeria in the 2-step method acc. to D.G.AL. and ISO 11290-1 (1996). EMD Merck KGaA, Damstadt, Germany, 2002. http://www.emdchemicals.com/analytics/Micro_Manual/TEDISdata/prods/1_10398_0500.html. Retrieved, March 9, 2009.

Applicants' arguments:

Applicants traverse this rejection for the same reasons set forth above in connection with the rejection over Berg and Pyle as combined.

Response:

Applicants' arguments are carefully considered but are not persuasive. The rejection of Berg in view of Pyle et al is set forth above. Applicants arguments with respect to the combination of Berg et al and Pyle et al have been carefully considered, are not persuasive and have been addressed above.

13. The rejection of claims 2-3 and 21-22 under 35 U.S.C. 103(a) as being unpatentable over Berg et al. WO 89/04372, 18th May 1989 (cited in IDS) and Pyle et al WO 95/31481 23 November 1995 (cited in IDS) as applied to claims 1, 4, 5, 6, 15 and 17 above, further in view of Strenkoski et al. (US 5,843,699 Dec. 1 1998) and Heck et al (US 3,704,204) Nov. 28, 1972) and Ray, Bibek (Injured Index and Pathogenic

Bacteria. 1989. CRC Press Inc. Boca Raton, Florida. Page 78) and Patel et al. (Journal of Food Protection, 1995 vol. 58, No. 3, p. 244-250) is maintained.

The combination of Berg et al and Pyle et al is set forth supra. Said combination does not teach enriching the microorganisms in a composition comprising sodium pyruvate, sodium thiosulfate and catalase.

Strenkoski et al teaches that recovering pathogenic microorganisms from a food samples involves a pre-enrichment wherein the food sample is enriched to restore injured bacterial cells to a stable physiological condition. Column 2 lines 53-55. Strenkoski et al also teaches that the pre-enrichment media can comprise a mildly selective inhibitor such as an antibiotic and that the selection of antibiotic inhibitor depends on the selected target microorganism. See column 6 lines 56-67 and column 7 lines 1-6. Strenkoski et al teaches at least one selective inhibitor or mixtures thereof can be added to the pre-enrichment media and teaches that sodium thiosulfate is a selective inhibitor. Column 7 lines 16-20 and 38-60.

Heck et al teaches sodium thiosulfate as a selective inhibitor at a concentration of about 0.03% -0.07% by weight. See column 2 lines 61-65.

Ray teaches that catalase and pyruvate also increases the count levels of Salmonella in enrichment and teaches that both of these reagents are able to react with or catalyze the decomposition of the toxic substance, hydrogen peroxide. See p. 78.

Patel et al teaches that since injured bacterial cells typically have increased sensitivity to hydrogen peroxide and superoxide radicals, due to decreased catalase and superoxide dismutase activities, the addition of pyruvate and catalase has been recommended to maximize repair and colony formation of injured S. aureus cells. See page 247 column 2 under discussion. Patel et al teaches supplementation of 1% pyruvate or 0.04% catalase to support an increased level of repair of heat injured L. monocytogenes. See page 247 column 2 under discussion. Patel also teaches that at 2.5 mg/mL (2.5 g/L) sodium pyruvate and 400 μg/ml catalase, injured cells under went resuscitation. See abstract. Patel et al also tested other concentrations of catalase and sodium pyruvate in table 1 p. 246. See whole of Patel.

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to add to the media of the selective enrichment step of the combination of Berg and Pyle, sodium pyruvate, sodium thiosulfate and catalase, thus resulting in the instant invention with a reasonable expectation of success. The motivation to do so is because Strenkoski et al teaches that sodium thiosulfate is a selective inhibitor for non-target microorganisms can be added to enrichment media used to restore injured bacterial cells and Patel and Ray et al teaches that sodium pyruvate and catalase are useful for maximizing repair and colony formation of injured bacterial cells (including Salmonella, S. aureus and Listeria) by acting on the toxic substance hydrogen peroxide and superoxide radicals. Furthermore, it would have been prima facie obvious to additionally add an antibiotic because Strenkoski et al teaches that the pre-enrichment media can comprise a mildly selective inhibitor such as an antibiotic and enrichment media can comprise at least one selective inhibitor or mixtures thereof.

As to the concentrations of sodium pyruvate, sodium thiosulfate and catalase in claims 2 and 22, MPEP 2144.05 states, "Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be *prima facie* obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also *Peterson*, 315 F.3d at 1330, 65 USPQ2d at 1382 ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); *In re Hoeschele*, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969) (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of

molecular weight or molar proportions.). For more recent cases applying this principle, see *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler,* 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997)." Heck et al teaches concentrations of sodium thiosulfate as a selective inhibitor at a concentration of about 0.03% -0.07% by weight and Patel et al teaches concentrations of catalase and sodium pyruvate that can be use to recover injured bacteria. Thus, it would have been prima facie obvious for one skilled in the art at the time the instant invention was made to arrive at workable ranges of sodium thiosulfate and sodium pyruvate and catalase as set forth in claims 2 and 22, with a reasonable expectation of success.

Applicants' arguments:

Applicants traverse this rejection for the same reasons set forth above in connection with the rejection over Berg and Pyle as combined.

Response:

Applicants' arguments are carefully considered but are not persuasive. The rejection of Berg in view of Pyle et al is set forth above. Applicants arguments with respect to the combination of Berg et al and Pyle et al have been carefully considered, are not persuasive and have been addressed above.

Status of Claims

Claims 1-10, 12-13, 15-17, 21 and 22 are rejected. Claims 18-20 are withdrawn. No claims allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to OLUWATOSIN OGUNBIYI whose telephone number is (571)272-9939. The examiner can normally be reached on M-F 5:30 am- 2:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms can be reached on 571-272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Oluwatosin Ogunbiyi/
Examiner, Art Unit 1645